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AWARD NUMBER: W81XWH-05-1-0475

TITLE: Restoration of Epithelial Polarity in Metastatic Tumors

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REPORT DATE: July 2006

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-07-2006	Final	1 Jul 2005 – 30 Jun 2006

4. TITLE AND SUBTITLE

Restoration of Epithelial Polarity in Metastatic Tumors

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0475

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Mount Sinai School of Medicine
New York, New York 10029-6574

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Malignant properties of breast cancer cells are known to depend on their altered polarity and adhesion properties. LGL genes function by controlling normal epithelial polarity and suppressing tumors in the fruit fly. The LGL proteins were also proposed to decrease the frequency of epithelial tumor formation in mammals. To test this idea, we monitored LGL expression in epithelial cells undergoing malignant transformation to correlate it with increased cell motility and altered cell shape. Whereas we found that LGL1 protein is present in most cell lines analyzed, we attempted to restore proper cell adhesion and epithelial organization of breast carcinoma cell lines by genetic manipulation of the LGL gene. We observed that the proliferation of carcinoma cell lines with increased expression of LGL1 has been suppressed, consistent with the hypothesis that LGL1 may function as a tumor suppressor. Our experiments may allow the design of physiologically relevant drugs against breast cancer.

15. SUBJECT TERMS
No subject terms provided.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	6	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION:

Breast cancer malignancy depends on altered polarity and adhesion properties of cancer cells. LGL genes are known to function by controlling normal epithelial polarity and suppressing tumors in the *Drosophila* fruit fly (Vasioukhin, 2006). The LGL proteins were also proposed to decrease the frequency of epithelial tumor formation in mammals. To test this idea, we proposed to monitor and manipulate LGL expression in epithelial cells undergoing malignant transformation and correlate it with altered cell shape and proliferation rate. The goal of the proposed study was to contribute to the development of new approaches of suppressing metastatic cell behavior by restoring LGL-dependent epithelial polarity and the design of physiologically relevant anti-cancer drugs.

BODY:

The project consisted of the following 3 tasks (specific aims):

1) To compare the localization of LGL in breast carcinoma cell lines.
2) To test the effects of LGL on breast carcinoma cell epithelial-mesenchymal transformation in vitro and on the regulation of metastatic behavior of mammary carcinomas in vivo.
3) To study the effects of Wnt ligands and their antagonists on LGL localization, EMT markers and cell motility in vitro and metastases in nude mice in vivo.

First, we have generated the required LGL cDNA constructs in vectors suitable for stable expression in mammalian cells and optimized our plasmid transfection protocols for carcinoma cell lines. As a positive control we used human embryonic kidney 293T cells and human breast carcinoma MCF7 cells. Regular transfection methods using a calcium phosphate technique was suboptimal for breast cancer lines that we tested, and we used electroporation for efficient plasmid transfer. Additionally, we have purified polyclonal antibodies to Lgl1 (Dollar et al., 2005) and used them successfully to detect the protein in cell lysates using western analysis (see Figure 1). We found Lgl1 expression in all 14 carcinoma cell lines that we tested (data not shown), suggesting that it is an essential gene and may be regulated at post-translational level.

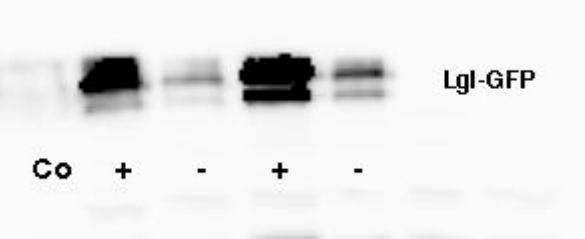

Figure 1. Tetracycline-inducible expression of Lgl1-GFP in transiently transfected human embryonic kidney 293T cells.

Although the initial attempts to establish stable transfectants using neomycin selection were successful, the transfected clones grew poorly and were difficult to maintain in culture. Slow proliferation of cells carrying Lgl1 plasmids is consistent with the hypothesis that Lgl is a tumor suppressor gene (Kuphal et

al., 2006). To overcome this problem of cell growth and evaluate Lgl1 effects on breast carcinomas, we had to develop an inducible system for overexpression. We decided to utilize pBIGi, a plasmid carrying a bi-directional tetracycline-inducible enhancer (Strathdee et al., 1999), which was driving both the Tet activator and the gene of interest (Lgl1). To allow an easy detection of the gene product in live cells, we constructed a green-fluorescent protein fusion. Transient transfection of this plasmid into cancer cells resulted in Tet-inducible expression of the gene. To date, we have successfully established several cell lines with this plasmid and are currently in the process of testing their growth rates upon induction of Lgl1 expression.

The next step of our research will be to develop in vivo assays for tumorigenicity and for metastases using developed stable cell lines and compare their properties upon induction of Lgl. We will test the tumorigenic potential of Tet-induced and uninduced cell lines in vivo by injecting them into immunodeficient 'nude' mice. These experiments are time consuming but they will provide new information for potential drug design.

We have also carried out task 3, to test the effects of Wnt proteins on Lgl1 localization and EMT markers. Wnt proteins did not detectably chance Lgl 1 localization when analyzed by immunofluorescence. These studies need to be repeated to achieve conclusive results.

KEY RESEARCH ACCOMPLISHMENTS:

- Construction of a Tet-inducible plasmid containing Lgl1 cDNA.
- Purification of a polyclonal affinity–purified anti-Lgl1 antibody.
- Generation of stable breast cancer cell lines carrying a Tet-inducible Lgl1 construct.
- Demonstration of anti-proliferative properties of Lgl1 in vitro.

REPORTABLE OUTCOMES:

We have designed new vectors developed stable breast cancer cell lines with inducible expression of Lgl1. We have demonstrated that cell lines expressing Lgl1 are growth-deficient, suggesting that Lgl is a tumor suppressor.

These data will be used to apply for a larger award that would allow us to complete this project and understand the role for Lgl in carcinoma cell prolliferation and metastatic cancers.

CONCLUSION:

Our results support the hypothesis that Lgl functions as a tumor suppressor in human cancer cell. Therefore, pharmacological activators of Lgl may be potentially used as anti-cancer drugs and should be tested for tumor inhibitory properties.

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APPENDICES:
None